

ABSTRACTS OF PAPERS

**Sixth Annual Albert L. Tester Memorial Symposium
3–4 April 1981¹**

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert Tester who, at the time of his death in 1974, was Senior Professor of Zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields within marine biology. Papers reporting original research on any aspect of biology are solicited from students at the University and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best papers by graduate students in the Department of Zoology. Papers are judged on quality, originality, and importance of research reported, as well as the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Dr. Stephen Jay Gould of Harvard University participated in the symposium.

¹ Manuscript accepted 15 May 1981.

**Behavioral Determinants of the Nocturnal Spacing Pattern of the
Yellow Tang *Zebrasoma flavescens* (Acanthuridae)**

PAUL ATKINS²

Field observations revealed day–night changes in the social organization and distribution of *Zebrasoma flavescens*, a diurnally active surgeonfish. This species occurs in a wide range of social group sizes (one to hundreds of individuals) while feeding during the day, and rests solitarily in shelter sites on the reef at night. The nocturnal dispersion pattern of individuals and the behavioral mechanisms that maintain this spacing pattern were investigated in the field by SCUBA

transect surveys and observations of tagged individuals, by nearest-neighbor analysis, and by laboratory experimentation.

Individuals repeatedly utilized the same shelter sites for periods of 6 months to 1 year. There was a positive correlation between an index of dispersion and population density within the apparently preferred nocturnal habitat (*Porites compressa*); individuals were evenly dispersed at high densities and aggregated at low densities. Social distance (i.e., mean nearest-neighbor distance) remained relatively constant at all densities.

During evening twilight, dramatic in-

² University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

creases occurred in the frequency of intra- and interspecific agonistic interactions. The probability of aggressive response by an individual to an approaching conspecific was correlated with the proximity of the conspecific to the individual's nocturnal shelter site. Laboratory experiments revealed the importance of social attraction for the shelter choice of individuals. Test fish preferred to shelter at sites in close proximity to conspecifics, and shelter choice could be altered by changing the position of a neighboring conspecific. The shelter choice of test fish maintained with conspecifics was more pre-

dictable and more temporally stable than that of controls maintained in social isolation.

The nocturnal spacing system is maintained by both attractive and repulsive social interactions. Aggregations are the result of social attraction, whereas the even spacing within aggregations results from territorial behavior that occurs only during a short period before and after sunset. The locations of individuals within a habitat and their continued residency at a shelter site appear to be largely a function of social rather than extrinsic, habitat-related factors.

Population Dynamics of *Dissodactylus mellitae* (Brachyura: Pinnotheridae) on Its Sand Dollar Host *Mellita quinquesperforata*³

JANICE L. BELL⁴

The population dynamics of the symbiotic crab *Dissodactylus mellitae* were determined by monthly collections of the crab's sand dollar host *Mellita quinquesperforata* in Georgetown County, South Carolina. Since the crab normally clings to the spines of its host but can be dislodged, individual sand dollars were carefully collected by SCUBA and subsequently examined in the laboratory.

Infestation of sand dollars ranged from zero to eight crabs per sand dollar with a mean of 0.97. Size frequency histograms indicated a 12- to 15-month life cycle for *Dissodactylus mellitae*. Most settlement occurred from May to August (early settlers), but recruitment of juveniles continued through September and October (late settlers). Early settlers grew close to full adult size by November, and overwintered at that size. Laboratory trials indicated that these animals

mated some time before February, perhaps in the fall. Females spawned in the spring (April–June), released their larvae, and disappeared shortly thereafter. Late settlers overwintered as small adults, continued growth the following spring, and spawned in August. Growth of all crabs ceased from November to March. All specimens had a hard carapace.

The sex ratio was 1:1 throughout the year. Though both males and females appeared to have similar growth patterns, females attained a larger maximum carapace width (4.82 mm for females versus 3.96 mm for males). Fecundity was constant over the spawning season (April–September), with a mean egg number of 206 ± 62 and a mean egg diameter of 281 μm . The number of clutches per female is unknown. The life cycle of *Dissodactylus mellitae* differs from the few other well-studied members of the family Pinnotheridae, most of which inhabit bivalves, maintain a soft carapace during much of their lives, have higher sexual dimorphism than *D. mellitae*, and release an order of magnitude more larvae per clutch.

³This research was supported by the Marine Science Program and the Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina.

⁴University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

Evidence for Functional Plasma Membrane-Associated RNA⁵

TERRY A. CARLSON⁶

Numerous investigations of cell membrane composition have reported a small fraction, usually less than 1 percent, of the isolated material to be ribonucleic acid (RNA). These results have often been attributed to cytoplasmic contamination during isolation. However, it has been shown that the electrophoretic mobility of various types of intact cells can be altered by their incubation with ribonuclease, indicating the presence of ribonuclease susceptible sites on the cell membrane. In addition, these sites were not detectable on lymphocytes from patients with chronic lymphocytic leukemia (CLL), a cancer of the lymphocyte system. Lymphocytes from these patients, and also from muscular dystrophy (MD) patients, have a reduced immunoglobulin G (IgG) antibody capping response, i.e., the movement of a fluorescently labeled ligand to one pole of the cell. It was therefore suggested that antibody capping may be a useful technique for studying membrane-associated RNA.

In this study, a standard capping pro-

cedure was used. Human lymphocytes were first isolated from a fresh blood sample and incubated in various concentrations of ribonuclease. Fluorescently labeled IgG antibody was then added, and cap formation was monitored by fluorescence microscopy. This procedure showed evidence of a correlation between decreased IgG capping of intact viable human lymphocytes and predigestion of the cells with increased concentrations of ribonuclease. At high concentrations, a minimum plateau of about 20 percent of labeled cells underwent capping, a level similar to those reported in CLL and MD patients.

Cap formation requires both energy metabolism and intact functional microtubules and microfilaments. It has been reported that in vitro microtubule formation of the mitotic spindle apparatus can be inhibited by ribonuclease treatment of isolated basal bodies, indicating that RNA is a necessary component of the spindle microtubule organizing center. Furthermore, it has been shown that cytoskeletal microtubule formation is diminished in fibroblasts of dystrophic chickens, suggesting a defect in the membrane microtubule organizing centers. The present study gives the first evidence that membrane-associated RNA may be involved in these organizing centers.

⁵ This research was supported by an American Cancer Society institutional award from the Cancer Center of Hawaii.

⁶ University of Hawaii, Department of Genetics, Honolulu, Hawaii 96822.

Settlement and Emergence in a Supratidal Hawaiian Limpet, *Cellana exarata* Reeve (Prosobranchia: Patellidae)⁷

GLADYS C. CORPUZ⁸

Cellana exarata Reeve, an archeogastropod limpet endemic to Hawaii, inhabits the upper

intertidal and supratidal zones of rocky shores. The species is largely restricted to the windward sides of the islands where surf action is high, resulting in high water aeration. In this study, two questions are addressed: (1) Are water aeration and/or water movement important factors that influence the site of settlement and metamorphosis in this species? (2) How do individuals

⁷ This research was supported by the State of Hawaii through a contract with Alison Kay; a National Institutes of Health grant to Michael G. Hadfield; a grant from the Hawaiian Malacological Society; and funds from the Department of Zoology, University of Hawaii.

⁸ University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

attain the specific zone (upper intertidal and supratidal) of their habitat?

To answer the first question, laboratory experiments were conducted on settlement and metamorphosis under various regimes including aeration, water depth, and substratum orientation. Settlement and metamorphosis were induced by exposure of metamorphically competent veligers to a hard substratum (glass) coated with algal film. In nonaerated cultures, settlement occurred near the water surface regardless of water movement. In aerated cultures, however, settlement occurred at all depths. The orientation of settling surfaces was not important when cultures were aerated. When unaerated, however, higher settlement occurred on vertical as opposed to horizontal surfaces, especially when the surfaces were partially exposed to the atmosphere.

The second question was addressed

through long-term observations of juveniles. In aerated aquaria, juveniles typically remained underwater until they were 4 wk old. At about 5 wk, they started to migrate upward beyond the water level (emergence). To determine whether the direction of water current influenced emergence, 2-month-old juveniles were subjected to a downward current. The results showed that emergence occurred at a faster rate in the presence of a downward current than in its absence.

It is hypothesized that in nature, settlement occurs on submerged rocks in waters highly aerated by wave and surf action. The upward migration of older juveniles probably results from physiological changes associated with increased efficiency in aerial respiration as opposed to aquatic respiration, increased resistance to desiccation, or other factors associated with age.

Age Determination and Growth of Three Species of Shallow-Water Carcharhinid Sharks in Hawaii⁹

MARK A. DECROSTA¹⁰

Three species of carcharhinid sharks are the most abundant top predators in the shallow-water (< 150 m) reef community in the northwestern Hawaiian Islands (NWHI), but very little is known about their growth and energetics. These sharks are the gray reef shark (*Carcharhinus amblyrhynchos*), Galapagos shark (*C. galapagensis*), and tiger shark (*Galeocerdo cuvier*). In order to develop a fisheries management plan for the NWHI, it is necessary to know the diet and energy needs of these top predators as well as their annual production, which is a function both of their population sizes and growth rates.

Vertebral samples from 62 gray reef, 45

Galapagos, and 28 tiger sharks were obtained. Vertebral centra were cleaned and stained with silver nitrate to allow the visualization and counting of presumed annual growth rings. These age data were used in conjunction with length and weight information to generate growth curves for the three species. The estimated von Bertalanffy growth curves for precaudal length (ℓ) were $\ell_t = 133.7$ cm $[1 - \exp(-0.294)(t + 0.869)]$ for the gray reef shark; $\ell_t = 230.0$ cm $[1 - \exp(-0.172)(t + 0.541)]$ for the Galapagos shark; and $\ell_t = 335.0$ cm $[1 - \exp(-0.155)(t + 0.619)]$ for the tiger shark. These curves correlate well with independent curves obtained from length frequency analysis of earlier fisheries statistics for Hawaiian sharks. The close correspondence between the two types of estimates of growth used verifies the annual nature of the vertebral rings in these sharks.

⁹This research was supported by the University of Hawaii Sea Grant Program, grant no. NI/R-4.

¹⁰University of Hawaii, Department of Oceanography, Honolulu, Hawaii 96822.

Growth information for these sharks is important because of their major role in the reef ecosystem in the NWHI. The Galapagos shark and the tiger shark are the two largest fishes in the habitat and therefore must have

relatively great energy needs. Because of the considerable impact these sharks must have on the other fauna, information on their biology should be used in management of this ecosystem.

The Influence of the Sea Grass *Zostera capricorni* on the Establishment of Soft-Bottom Benthic Communities: An Experimental Study¹¹

RACHEL FITZHARDINGE¹²

Experiments to investigate the effect of *Zostera capricorni* on the establishment of soft-bottom benthic communities were performed in Smiths Lake, New South Wales, Australia. Two factors associated with the presence of sea grasses were studied. The first factor was the increased spatial heterogeneity provided by the shoots of the plants, and the second, the increased food supply for detrital feeders resulting from the decaying leaves of the plants.

Experimental faunal assemblages were established in buckets containing one of five treatments. One treatment (control) contained sediment only. Two treatments contained sediment and preaged *Zostera* detritus in different amounts (35 g m^{-2} and 70 g m^{-2}). The fourth treatment contained sediment and artificial sea grass in the form of plastic strips stapled onto a plastic mesh frame buried below the level of the sediment surface. The fifth treatment contained sediment, artificial sea grass, and sea grass detritus (35 g m^{-2}). The experiment was laid out in two tied Latin squares. One was in a

Zostera bed, the other one about 20 m away in an unvegetated sand flat. The experiment was terminated after 3 months, and at this time, five replicate core samples were taken from both habitats.

Within samples from a habitat, significant treatment effects were found in the number of species per sample as well as the number of individuals per sample for certain species. Generally, the highest values for these measurements were found when artificial sea grass was added. Differences were also found in the abundance of certain taxa for a given treatment type between habitats. This phenomenon was most obvious in treatments with artificial sea grass. These samples from the sand flat became dominated by "opportunistic" species such as *Styela plicata* (Chordata, Ascideacea) and *Megamphopus* sp. (Crustacea, Aphipoda). These species occurred at much lower densities in equivalent treatments within the *Zostera* bed and in core samples taken from either habitat.

The results of these experiments suggest that both the nature of the surrounding habitat and the increase in spatial heterogeneity associated with the sea grass shoots can greatly influence the fauna that become established in an area. The addition of sea grass detritus did not significantly change the number of individuals that became established in the experimental containers in either habitat.

¹¹ This research was performed while I was a graduate student at the University of New South Wales. It was partly funded by grant 12-045-16 to R. J. MacIntyre, and partly by the School of Zoology, University of New South Wales.

¹² University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

Evolutionary and Functional Relationships among Di- and Tripeptidases of Fish¹³

LLOYD FRICK¹⁴

The evolutionary and functional relationships among the peptidases of fishes were investigated. Five isozymes (PEP A, B, C, D, and S) of the tuna *Katsuwonis pelamis* were purified and characterized biochemically. All these isozymes except PEP D were also characterized immunologically. The results of these analyses suggest that the five peptidases (each presumably encoded by a separate structural gene) are not closely related, since they do not share immunological determinants, and are affected differentially by metals, metal chelators, and mercurials. Homologies of fish peptidases with their human counterparts are certain only in the case of the PEP A's; anti-tuna PEP A sera cross-react with the human form. Fish and human PEP B, D, and S are also probably homologous, although fish PEP B is a dimer, whereas the human enzyme is a monomer. Homology between the two PEP C's is less certain because of differences in both subunit composition and substrate specificities.

Clues to the functional roles of these enzymes can be gleaned from the data on their tissue distributions and substrate specificities. PEP A, B, D, and S have tightly correlated tissue distributions, which suggests involvement in similar areas of peptide metabolism. The substrate overlap of PEP A, B, and D is minimal, but PEP S overlaps to some extent with both PEP A and B. All four of these isozymes are present at highest levels in organs such as intestine and kidney, which are heavily involved in the catabolism of peptides. This implies that these enzymes are specialized with respect to substrate specificity, and that they are together sufficient for the complete hydrolysis of cytoplasmic di- and tripeptides generated as a result of extra- or intercellular protein and peptide catabolism. The role of PEP C is mysterious, because it is not associated with tissues that have a particularly active or unique peptide metabolism. PEP C occurs mainly in the brain and eye of most fishes. Its substrate specificity overlaps almost completely with that of PEP A. Its physiological role may be to take the place of PEP A in tissues for which PEP A is unsuited.

¹³This research was supported by a fellowship from the Jesse Smith Noyes Foundation.

¹⁴University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

Examination of G Bands by Differential Staining¹⁵

JODY E. GRIFFITH¹⁶

In 1970, it was discovered that human chromosomes show a reproducible banding pattern when stained with DNA-binding

fluorescent agents. Since that time, a large number of techniques have been devised to produce similar staining patterns. Giemsa banding of human chromosomes has proved to be a useful tool for karyotyping in clinical genetics by facilitating the detection of deletions, trisomies, and other disease-related chromosomal abnormalities. Banding is also

¹⁵This research was supported in part by National Science Foundation grant PCM 70-16350.

¹⁶University of Hawaii, Department of Genetics, Honolulu, Hawaii 96822.

an essential part of gene mapping and localization of specific genes by in situ hybridization techniques.

Numerous investigators have been working to understand the molecular basis for banding, and an equivalent number of hypotheses have been presented. The three major hypotheses maintain that bands, or darkly stained areas, represent (1) the distribution of DNA-associated nonhistone proteins; (2) areas of heterochromatin as opposed to interband regions of euchromatin; or (3) the distribution of free protein.

The present study is based on the premise that G bands are regions of DNA-associated nonhistone proteins, whereas interbands are areas of reduced protein-DNA association. Since all Giemsa-banding techniques involve protein denaturation (e.g., trypsin digestion), it is conceivable that DNA in the banded regions is complexed with nonhistone protein to a greater extent than DNA in the interbands. When untreated chromosomes are stained with Giemsa, faint bands are present.

This supports the hypothesis that bands are the delineation of a preexisting structure.

To test further the nonhistone protein hypothesis, human metaphase chromosomes isolated from peripheral blood were subjected to G-banding procedures and stained with a protein-specific dye. These chromosome preparations were then viewed with light microscopy and scanning electron microscopy (SEM). Chromosomes digested with trypsin and subsequently stained with Coomassie Brilliant Blue R-250, showed a banding pattern similar to that of G bands. SEM micrographs showed areas of extensive digestion and areas of preserved morphology corresponding to interbands and bands, respectively. Similar results were obtained by comparing light microscope and SEM micrographs of Giemsa-stained chromosomes. These data support the hypothesis that differential organization of nonhistone protein-DNA complexes is responsible for patterns of staining in G-banded chromosomes.

Comparative Reproductive Biology of Hawaiian Vermetid Gastropods

CAROL N. HOPPER¹⁷

Numerous studies suggest that closely related invertebrate species with different body sizes have different life history patterns. The present study compares reproductive patterns of small and large species of vermetid gastropods. Sex and degree of gonadal development were determined for individuals of three Hawaiian vermetid species, each collected at monthly intervals for at least 1 year. Individual fecundity and pattern of embryo production were determined for each species by examining embryos from brooding females. *Dendropoma gregaria*, a small (≤ 101 mg), gregarious species with direct development, produced few embryos per brood. The level

of population reproductive activity was high in this species (up to 38 percent of the population consisted of brooding females). Females produced seven broods of embryos per year, and while annual individual fecundity was low, fecundity per milligram of body weight was high. *Dendropoma platypus* is a non-gregarious vermetid of intermediate size (≤ 754 mg) with direct development and an intermediate number of embryos per brood. Brooding females were found in all months of the year, but the level of population reproductive activity was low (≤ 26 percent brooding females), and individual females produced only three broods per year. *Vermetus alii* is a large (≤ 2224 mg), non-gregarious vermetid with indirect development and a large number of embryos per

¹⁷ University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

brood. Population reproductive activity was low (≤ 25 percent brooding females) over the 7-month reproductive season of this species, and individual females produced three broods per year. In the two larger vermetid species, individual females had high annual fecundities, but low fecundities per milligram of body weight. Life history pat-

terns of these vermetids can be summarized: *D. gregaria* begins reproducing at an early age, produces several small broods per year, and probably has a short life-span; *D. platypus* and *V. alii* begin reproducing at later ages, produce few large broods per year, and individuals probably live longer.

The Pattern of Gametogenesis in *Cellana exarata* (Prosobranchia: Patellidae)

LLOYD K. ITO¹⁸

Gametogenesis in the Hawaiian limpet *Cellana exarata* Reeve was investigated during one of the two spawning seasons in 1980. Adults were collected weekly from April to June, the gonads separated from the rest of the body, fixed in 10 percent formalin, and prepared for histological examination. Analysis of oocytes in various stages of development revealed five distinct stages based on oocyte size; absence, presence, and thickness of the outer covering (the chorion); and the oocyte's position relative to the ovary wall. Development progresses from an oocyte 1 μm in diameter and attached to the ovary wall to an unattached oocyte about 115 μm in diameter, ready for spawning. The last stage of development is characterized by the presence of a chorion 1.2–5.0 μm thick. The smallest oocytes were less than 37.5 μm in diameter and always outnumbered the large oocytes. The largest oocytes were about 115–125 μm in diameter and were negatively correlated with the number of small oocytes. Oocytes that may be spawned include those with diameters greater than 75 μm .

Although spermatogenesis was not ana-

lyzed as thoroughly as was oogenesis, two stages were obvious: (1) when the developing testis contained mostly maturing spermatogonia; and (2) when the spawning testis was predominated by mature spermatozoa. Males appear to produce and release spermatozoa throughout the spawning season.

To verify the data obtained for April–June, animals were collected monthly during the second spawning season in 1980, from September to November. The results of the analyses of these gonads were not different from those of the April–June collections.

Several conclusions can be drawn from the data: (1) Throughout each spawning season, a fraction of both sexes is undergoing gametogenesis and/or spawning. (2) Within the gonad of any one individual, gametogenesis is synchronous. (3) Throughout the spawning season, new immature oocytes develop at approximately the same rate as mature ova are spawned or resorbed. (4) As the spawning season progresses, a smaller volume of oocytes is spawned per female and the percentage of normal development decreases. The data suggest that normal development of spawned ova requires that at least 10 percent of the oocytes in the ovary be at least 75 μm in diameter.

¹⁸ University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

The Mokauea Fishpond Project¹⁹

DWIGHT H. KONDO, SHELLEY CHOY, MICHAEL MARUYAMA, and NORMAN NAKAMURA²⁰

Mokauea fishpond is a 2.2-acre Hawaiian-style fishpond on Mokauea Island in Ke'ehi Lagoon, O'ahu. It is part of an effort to rehabilitate a subsistence-level fishing village at Mokauea. Since February 1980, construction and maintenance of the pond and gate system have been completed with the help of University of Hawaii students and volunteers from the community. Two species traditionally raised in Hawaiian fish ponds, the mullet (*Mugil cephalus*) and milkfish or *awa* (*Chanos chanos*), are being cultured. The *mākāhā* (gate system) is the primary tool used in the management of the pond. The unique design of the sluice gate system allows optimum management of water quality. Physical, chemical, and biological parameters have been mea-

sured on a routine schedule and have provided a basis for developing a management strategy that will be taught to the residents and members of the Mokauea Fishermen's Association. In November 1980 some 180 *awa* ranging from 20 to 30 cm in length were released in the pond. Project members have conducted two samplings of the fish growth since that time. Data will be compared to information gathered by other researchers working at the Oceanic Institute on the same stock. The *awa* in the Mokauea pond have been feeding on the macroalgae, phytoplankton, and zooplankton that grow naturally in the pond. Since the early part of this year, natural recruitment of mullet fry to the pond has occurred through the *mākāhā*. Project members have also been stocking the pond with mullet fry captured in the surrounding waters. Future activities for Mokauea fishpond include new options for pond management to provide a higher level of production and stepping up our resident training program.

¹⁹This project has received funding since September 1980 from the Aquaculture Development Program, the Marine Affairs Coordinator of the State of Hawaii, and the University of Hawaii Sea Grant College Program.

²⁰University of Hawaii, Marine Option Program, Honolulu, Hawaii 96822.

Molt-Related Changes in Transmural Na Transport in Freshwater Prawn Intestine²¹

LINDA K. KULLAMA²²

It is well established that various portions of the digestive tract take part in salt and water regulation in crustaceans. In intermolt *Macrobrachium rosenbergii*, the freshwater prawn, there is a net movement of Na, Cl, and H₂O across the midgut in the direction lumen to hemolymph. In addition, net H₂O movement varies with the molt cycle. Transmural Na transport across the midgut

throughout the molt cycle was examined using an in vitro perfusion technique. Mucosal-to-serosal flux, J_{ms}^{Na} , was determined by perfusing the midgut with ²²Na-ringer and periodically sampling the serosal bath. The reciprocal to this experiment, i.e., adding ²²Na-ringer to the serosal compartment and sampling the perfusate effluent, was done to determine serosal-to-mucosal flux, J_{sm}^{Na} . The net flux was calculated as the difference between the two unidirectional fluxes. Fluxes (mean micromoles per square centimeter per hour) and probabilities that the net flux does not differ from zero are presented in the table.

²¹This research was supported by National Science Foundation grant PCM 76-84105.

²²University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

MOLT STAGE	TRANSMURAL SODIUM FLUX (μ moles/cm ² /hr \pm SEM)		
	J_{ms}^{Na}	J_{sm}^{Na}	J_{net}^{Na}
Intermolt (C)	6.4 \pm 0.36	4.0 \pm 0.32	2.4 ($p < 0.001$)
Premolt (D ₃)	6.9 \pm 0.41	4.3 \pm 0.99	2.6 ($p < 0.05$)
Late premolt (D ₃ ^{'''})	3.2 \pm 0.57	2.1 \pm 0.53	1.1 (n.s.)
Immediate postmolt (A)	2.1 \pm 0.37	1.2 \pm 0.09	0.87 ($p < 0.001$)
Late postmolt (B)	3.5 \pm 0.48	2.5 \pm 0.36	1.0 (n.s.)

In all molt stages, J_{ms}^{Na} exceeded J_{sm}^{Na} , although the net fluxes were not all significantly different from zero. Both the absolute values of the unidirectional fluxes and the differences between them decrease beginning in late premolt (< 48 hr prior to molt), attain their minima in early postmolt, and rise toward intermolt values in late postmolt individuals. Previous work has indicated that J_{sm}^{Na} provides a good estimate of paracellular permeability; the low J_{sm}^{Na} variability seen in A animals could be an indication of the influence of molt-related factors on passive permeability characteristics. Changes in J_{ms}^{Na} fluxes may be indicative of effects on cell membrane permeability and/or ion pump

activity. Published water fluxes correlate well with J_{net}^{Na} over the molt cycle and suggest that the two may be coupled. Analysis of serum properties at corresponding molt stages showed two general trends: (1) a 5–10 percent increase in osmolarity with a concomitant increase in Na and Cl in premolt animals; and (2) a similar-sized decrease in these factors in immediate postmolt individuals. Evidence thus far points to a possible regulatory role, probably secondary, of the midgut in salt and water balance in *Macrobrachium rosenbergii*, with a suggestion of molt-related effects on both transcellular and paracellular transport parameters.

Hepatopancreatic Sugar Transport in the Atlantic Lobster *Homarus americanus*²³

ELIZABETH A. MONCKTON²⁴

³H-Methyl-D-glucose transport across basolateral membranes of the Atlantic lobster (*Homarus americanus*) hepatopancreas was investigated. Tissue respiration rate at 15°C in lobster saline containing 1 mM methyl glucose was $57 \pm 3 \mu\text{l O}_2 \text{ g}^{-1} \text{ wet weight hr}^{-1}$, similar to published oxygen consumption values for crustacean intestine. Fragments of hepatopancreas were incubated at 15°C in lobster saline containing various test

solutes for periods ranging from 60 to 180 min and the effects of these solutes on tissue methyl glucose accumulation was ascertained. Glucose analog uptake was nonconcentrative, approaching a tissue/medium concentration ratio of only unity after 3 hr of incubation. Phloretin (0.1 and 0.5 mM), sodium azide (10 mM), and ouabain (1 mM) did not significantly ($p > 0.05$) influence methyl glucose accumulation, whereas phloridzin (0.5 and 1.0 mM) and D-glucose (25 and 50 mM) significantly ($p < 0.01$) reduced sugar uptake. Methyl glucose influx, examined using 10-min incubations of tissue fragments, was a hyperbolic function of exog-

²³This research was supported by National Science Foundation grant PCM 76-84105.

²⁴University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

enous sugar concentration (after extracellular space correction using ^3H -mannitol), and kinetic constants were calculated from these influx data ($K_t = 11.4 \text{ mM}$; $J_{\text{MG}}^{\text{max}} = 5400 \text{ nmole g}^{-1} \text{ hr}^{-1}$). Thin-layer chromatograms of tissue fragment ethanol extracts following a 60-min ^3H -methyl-D-glucose incubation exhibited two major activity peaks, one corre-

sponded with a ^3H -methyl-D-glucose standard, and the other appeared to be a methyl glucose phosphate (sensitive to alkaline phosphatase). Results suggest that basolateral sugar uptake occurs by a facilitated diffusion system which is tightly coupled to intracellular phosphorylation.

A New Record of Slipper Lobster *Scyllarides* in Hawaii

TERRANCE D. MORIN²⁵

Seven species of slipper lobsters (Decapoda, Scyllaridae) are reported from Hawaiian waters, two for the first time, namely *Scyllarus vitiensis* (Dana) and *Scyllarides haanii* (deHaan).

The genus *Scyllarides* is represented by two species, previously confused as *S. squamosus* (H. Milne-Edwards), the only recorded *Scyllarides* from Hawaii. Close inspection of morphological characteristics of a juvenile *Scyllarides* sp. maintained at the Waikiki Aquarium revealed several differences from preserved and live adult specimens of *S.*

squamosus. Collections at Kure Atoll, in the northwestern Hawaiian Islands, provided live specimens of juvenile *S. squamosus* which were used for a more direct comparison with the juvenile *Scyllarides* sp. from the Aquarium. Analysis of morphological characteristics of these and other previously collected specimens revealed that two distinct species of *Scyllarides* do, indeed, occur in Hawaii. Electrophoretic studies of specimens from Kure Atoll confirmed the existence of two separate species. A survey of the published literature indicates that the second species is *S. haanii* (deHaan). The characteristics of the two species of *Scyllarides* are illustrated and compared.

²⁵ University of Hawaii, Waikiki Aquarium, Honolulu, Hawaii 96815, and Pacific Islands Program, Honolulu, Hawaii 96822.

Trap Studies on Captive Populations of the Spiny Lobster *Panulirus marginatus*²⁶

LINDA M. PAUL²⁷

Population densities of the endemic spiny lobster *Panulirus marginatus* are commonly estimated using catch/effort data. In order to

detect any bias associated with traps, a captive population of known density, sizes, and sex ratios was exposed to a standard California-type, two-chambered, wire-mesh trap. The test populations consisted of equal numbers of lobsters in each of several size classes. The ratio of males to females was 3 : 2. Two different population densities were

²⁶ This work was supported in part by the National Marine Fisheries Service.

²⁷ University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

tested: 3.1 lobsters per square meter and 1.7 lobsters per square meter. Traps were set for one night at 14-day intervals for a total of nine trap nights. The percent catch of all lobsters in the test population over 6.6 cm carapace length (CL) was high density: males 17 percent, females 30 percent; low density: males 31 percent, females 27 percent.

Another aspect of this study was to determine what percentage of sublegal lobsters (less than 8.25 cm CL) would leave a trap fitted with an escape panel, and if the catch of legal animals in such a trap would increase. A standard trap was covered with a fine wire mesh and fitted with one of three types of escape panels across the end of the inner chamber: a single-bar gap, a single-centered gap, or a mesh of 15 gaps. Gap width measured 6.0 cm, which would permit an 8.15-cm CL lobster to escape. A random selection of 26 lobsters was loaded into an unbaited trap and left down one night on eight different occasions. The bar gap permitted the highest sublegal escapement at 60 percent; the mesh and single gaps yielded 50 percent escapement. Of those loaded, 10 percent more females than males were retained. Thirty-three percent of the catch consisted of lobsters

not previously loaded.

Molt records indicate that molt interval increases with size. On the average, 5–6-cm CL lobsters molted 5.6 times per year, 6–7-cm CL lobsters molted 5.3 times, 7–8-cm CL lobsters molted 5.1 times, and 8–9-cm CL lobsters molted 4.8 times. The lobsters were observed to be inactive and were only rarely trapped 7–9 days before and after molting. This factor results in lobsters over 7.0 cm CL being unavailable to the fishery for about 20 percent of the year.

The lobster's reaction to a baited trap set at one of four different orientations to the current was observed for 1–2 hr at twilight. Lobsters were labeled with large epoxy putty numbers on their dorsal carapace so that individuals could be identified. Lobsters usually approached the trap directly downstream from the bait container, which resulted in those traps with the container positioned upstream to the trap entrance having a higher catch rate.

Results of this study indicate that these traps exhibit biases that may affect the accuracy of population estimates based on catch/effort data. Escape panels may be a reasonable method of sublegal stock conservation.

Sexual Differences in Parental Ability and Reproductive Success in a Monogamous African Cichlid

ANNE PHELPS²⁸

Monogamy in fish is relatively rare. The purpose of this study was to test some current predictions for parental role division and relative reproductive success in the monogamous pair. The hypotheses to be tested were: (1) that females alone should be more successful than males alone; (2) that both parents together should be at least twice as successful as either parent alone; and (3) that either sex would stay with the fry once its

mate was removed (since no alternate mates were available, and the presence of predators made it unlikely fry would survive alone). Three wading pools measuring 1.65 m in diameter and 50 cm deep each contained one pair of *Pelviachromis kribensis* adults and three predator *Hemichromis bimaculatus* subadults. Inverted flower pots were provided for nest sites. As a measure of reproductive success, eggs were counted when spawning was complete, and fry were removed and counted at the end of each reproductive sequence. Sequential trials with the parents

²⁸ University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

tested together, the females tested alone, and the males tested alone produced a measure of average success. If all eggs resulted in surviving fry, success would be 100 percent (1.0). Substantiating the first two hypotheses, the average pair success (0.22) was more than twice the average success of either the female alone (0.077) or the male alone (0.00). However, contrary to prediction (3), males

refused to care for eggs and deserted after the female of a pair was removed. Thus, there is a greater role division in this African cichlid than is usually seen in South American monogamous cichlids, and the results are commensurate with the greater tendency for polygamy seen in the majority of the African cichlids.

Experimental Evidence for Stimulation and Inhibition of Sex Change in the Saddleback Wrasse *Thalassoma duperrey*

ROBERT M. ROSS²⁹

The saddleback wrasse *Thalassoma duperrey* is a diandric protogynous hermaphrodite whose adults consist of both initial-phase (IP) females and males and terminal-phase (TP) males. Females and IP males spawn in groups, while females and TP males spawn in pairs on the reef. To determine the proximate causes of sex change at the level of the individual, 12 experiments were conducted in outdoor pens. Adult wrasses were subjected to various social regimes using size, sex, coloration (phase), and density as independent variables. Single isolated females did not change sex, while the larger individuals of female pairs did, regardless of absolute size. These results were not affected by the amount of space available to the fish. Females paired with smaller IP males also changed sex, but those paired with smaller congeneric wrasses did not. A tactile barrier

(12.5-mm² wire-mesh screen) separating two females did not prevent sex change. However, a tactile visual barrier (two louvered panels separated by fine-mesh polyethylene screen) placed between two females reduced the percentage of sex changes significantly. Neither the presence of IP nor TP males was able to suppress sex change in the larger individual of a female pair opposite a tactile barrier. In the case of female threesomes, only the largest female changed sex, demonstrating suppression of sex change in the intermediate-sized fish.

These results show that sex change is socially controlled in *Thalassoma duperrey*, requiring stimulation from smaller conspecifics while showing inhibition in the presence of larger conspecifics. I interpret these processes (stimulation and inhibition) to be part of a finely tuned mechanism that determines the reproductive success associated with individual sex change in nonharemic, promiscuous species.

²⁹ University of Hawaii, Hawaii Institute of Marine Biology and Department of Zoology, Honolulu, Hawaii 96822.

The Diet of Goatfishes (Mullidae) from Midway Islands³⁰

CAROL T. SORDEN³¹

The diets of three species of goatfish (Family: Mullidae) were examined using fish from Midway Islands, northwestern Hawaiian Islands. The data will be used in resource management planning for commercial fisheries in the northwestern Hawaiian Islands. Fishes were collected by spearing, the gut cavities were injected immediately with 100 percent formalin, and whole fish were preserved in 10 percent formalin. In the laboratory, the fish were weighed, measured, and sexed, and guts were removed. Prey items in the gut contents were identified and counted, and volume displacement was taken. An index of relative importance for each prey item was calculated.

Parupeneus multifasciatus appears to be a specialized feeder with more than 50 percent of the specimens having only one or two prey taxa in their gut contents. Xanthid crabs and shrimps comprised the major component of the diet of this species. *Parupeneus pleurostigma* and *Mulloidichthys flavolineatus* are

more generalized feeders, eating a number of different taxa. Xanthid crabs were the most important prey for *P. pleurostigma*, but this species also eats considerable amounts of polychaetes, shrimps, portunid crabs, crab megalops, and prosobranch gastropod mollusks. Polychaetes were found in the gut contents of all *M. flavolineatus* examined. Also important in the diet of *M. flavolineatus* are bivalve and opisthobranch mollusks, xanthid crabs, gammaridean amphipods, crab megalops, isopods, and tanaids.

There is a 67 percent overlap in the diets of *Parupeneus pleurostigma* and *P. multifasciatus*, with no dietary overlap between *M. flavolineatus* and *P. pleurostigma* or *P. multifasciatus*. Xanthid crabs are the major contributor to the overlap between the two *Parupeneus* species, and there appears to be no significant difference in the size of crabs eaten by the two species.

The diet of *Parupeneus multifasciatus* from Midway is very similar to that of the same species from Kona, Hawaii, but there are differences in the diet of *M. flavolineatus* between Midway and Kona, and between Midway and the Marshall Islands.

³⁰This research was supported by the University of Hawaii Sea Grant Program, grant no. NI/R-4.

³¹University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

Magnetic Sensitivity in Yellowfin Tuna *Thunnus albacares*³²

MICHAEL WALKER³³

In the open ocean the earth's magnetic field could be an important navigational cue for migrating animals. However, it is unknown whether pelagic marine animals can detect and use either the compass or map

information available from the geomagnetic field. Here, I report that yellowfin tuna (*Thunnus albacares*) can be conditioned to discriminate between altered and normal magnetic fields. The mechanism by which this fish detects the magnetic field may be based on magnetic material found in the skull.

To provide magnetic field control for conditioning experiments, 100 turns of 18AWG wire were wound around a 5.5-m-

³²This work was partly funded by a graduate study award from the East-West Center.

³³University of Hawaii, Department of Zoology, Honolulu, Hawaii 96822.

diameter wooden tank and a 6-m-diameter fiberglass tank. A current of 1 A in the coils produced a vertically oriented field stimulus of 0.1–0.50 Oe in the tanks. Three fish were trained using operant conditioning to discriminate between the normal geomagnetic field and altered fields induced by the coils around the experimental tanks. After about 60 trials, the fish reliably discriminated the normal from the altered field. Discrimination was unaffected by presenting the altered field gradually or instantaneously, suggesting that the fish were not using an electrical sense to detect an electrical field induced by a rapidly

changing magnetic field.

A possible mechanism for magnetic field detection by the tuna involves specialized sensory organelles using magnetic crystals. Using the superconducting (SQUID) magnetometer at the Hawaii Institute of Geophysics, I found ferromagnetic inclusions in the skulls of several tuna species. In the yellowfin, these inclusions were consistently localized within the ethmoid bone complex, suggesting that the magnetic material may be the basis for a magnetic sensory system in these fish.

The Isolation of Temperature-Sensitive Amino Acid Transport Mutants in a Transformed Human Cell Line

LESTER YIM³⁴

A transformed human cell line was grown until confluent in sixteen 100-mm² Corning culture dishes. All dishes were exposed to ultraviolet radiation at a distance of 80 cm for 15 sec. On the ninth day of incubation following ultraviolet mutagenesis, the cells were raised to the nonpermissive temperature of 39°C for 2 hr. A poisonous analog, *p*-fluorophenylalanine (PFPH), was introduced at this point at a concentration shown previously to be lethal to this human cell line. After 24 hr at 39°C in the presence of PFPH, the cells were washed at 39°C and given normal media with fetal calf serum (10 percent). A 24-hr incubation followed at the permissive temperature of 35°C. The process was repeated, beginning with the reintroduction of PFPH. Following this procedure, the cells were allowed to recover for 24 hr.

Cells were removed from the culture dishes and cloned as individual lines. Nine lines were isolated in this manner. One line has been tested for transport of *L*-phenylalanine.

Mutant line E6 was plated on 60-mm² Corning culture dishes. Before confluency was reached, the cells were placed in a water bath set at either 39°C (nonpermissive) or 35°C (permissive). The cells were washed five times with 2 ml water bath-temperature buffered saline. A 30-min incubation followed, after which the cells were exposed for 2 min to various concentrations of ³H-phenylalanine. Cells were then washed five times with 2 ml ice-cold buffered saline. Two milliliters of cold trichloroacetic acid (TCA) were then added to lyse cells and precipitate the protein. A 0.1-ml sample of the resulting TCA-soluble fraction was counted in a liquid scintillation counter.

The TCA-insoluble fraction from the cells was solubilized in 1 ml 1 *N* NaOH, and a 0.1-ml sample of this solution was counted in a scintillation counter. Following this, a Lowry protein determination was performed. These data were used to determine the rate of transport of *L*-phenylalanine per milligram of protein.

Using this approach, clone E6 has been shown to be a transport mutant at the non-permissive temperature of 39°C, but to

³⁴ University of Hawaii, Department of Genetics, Honolulu, Hawaii 96822.

transport at parental rates at 35°C. Further work on E6 and the other eight mutant lines will be used to determine the exact nature of the defect in these resistant cells. Comple-

mentation analyses and isolation of the altered transport protein(s) will further increase our knowledge of the genetic nature of amino acid transport.